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Reading

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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DEC 27 1983

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Mr. Robert Taylor
Registration Division (TS-767)

SUBJECT: Mouse Oncogenicity Study with Garlon
#464-554 - PP #1F2508 CAS 882-I

I have attached and concur with Dr. Kasza's analysis of the Garlon mouse data.

William L. Burnam, Chief
Toxicology Branch
Hazard Evaluation Division

Attachment

cc:
WWoodrow

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

December 20, 1983

TO: William Burnam
Chief of Toxicology Branch, TS-769.

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

FROM: Louis Kasza, D.V.M., Ph.D.
Toxicology Branch, Pathologist, TS-769 Louis Kasza

SUBJECT: Reevaluation of Lung Tumors in CDF/Cox Mouse, Treated with "Garlon"
EPA Regulation #464-546, 464-554, PP # 1F2508

SUMMARY

Giving careful evaluation of the results of different data and measuring the weight of differences between control and test groups, and considering the results of historical and concurrent control data, it can be concluded that the oncogenic potential of "Garlon" can not be substantiated.

INTRODUCTION

In the submitted report by Dow Chemical Company, the neoplastic and hyperplastic lesions were summarized in the following two tables:

TABLE 1

Neoplastic Respiratory System Lesion in Mice Fed Dowco 233*

MALES Tumor Type	Number Tumors/No. Examined Concurrent Control	Concurrent Control Proportional Basis	Study Control	Treated Animals 24 ppm	80 ppm	240
Adenomas	8/100	4/50	6/50	14/48	11/49	15/
Adenocarcinomas	25/100	13/50	3/50	2/48	6/49	4/
Total Tumors/ No. Examined	33/100	17/50	9/50	16/48	17/49	19/
FEMALES						
Adenomas	8/100	4/50	5/50	9/50	6/48	14/5
Adenocarcinomas	17/100	9/50	2/50	3/50	4/48	1/5
Total Tumors/ No. Examined	25/100	13/50	7/50	12/50	10/48	15/5

*The data do not indicate that all lung tumors were found at terminal sacrifice; animals dying naturally or which are subjected to moribund sacrifice must have been included in Table 1 data.

The results of the present study indicate an increased incidence of neoplasms in test groups in comparison to the study control group.

The statistical analysis of the data of proliferative changes (hyperplasia, adenoma and carcinoma) is summarized in the following table:

TABLE 2

Analysis of the Incidence of Lung Neoplasms in Mice Fed Dowco 233 in the Diet

Number of Tumor-Bearing Animals/ Number of Animals Examined

	MALES Dose (ppm in Diet)				FEMALES Dose (ppm in Diet)			
	0	24	80	240	0	24	80	240
Alveolar Cell Hyperplasia	1/50	0/48	2/49	1/49	6/50	0/50	1/48	3/50
Alveolar Adenoma	6/50	14/48*	11/49	15/49*	5/50	9/50	6/48	14/50*
		p=0.0310		p=0.0210				p=0.198*
Alveolar Adenocarcinoma	3/50	2/48	6/49	4/49	2/50	3/50	4/48	1/50
Combined Neoplasms	9/50	16/48	17/49*	19/49*	7/50	12/50	10/50	15/50*
			p=0.0481	p=0.187				p=0.448

*Indication of a significant increase in tumor incidence when compared to the Dowco 233 control group ($p = < 0.05$).

MATERIALS AND METHODS

As a result of the Company's response, in our Memorandum of August 11, 1983, I suggested the following:

> "In the absence of historical data for the CDF_1/Cox mouse, Dr. Louis Kasza (Toxicology Branch, Pathologist) recommended that Dow Chemical should provide the Agency with at least 5 additional experimental control records and conduct a literature search to explain the incidence of lung tumors in the CDF_1/Cox mouse, prior to making final conclusions regarding lung tumor incidence for these mice, as requested on page 13 of this communication. Dr. Kasza also recommended that proper justification for accepting the statement that concurrent control animals were kept under identical conditions than study control animals, and that study and concurrent control animals came from the same source should be made prior to a final conclusion on mouse oncogenicity."

3

RESULTS

On October 10, 1983, we agreed to meet the Company's pathologists (Drs. Warren and Kociba) to discuss the problem of lung tumors in that strain of mice they used. Other professional people from the Company were also present. From the EPA, Mr. Robert J. Taylor (TS-767C) and myself were present. We discussed the genetical background of CDF/Cox mouse strain and the circumstances of the limited knowledge of the background of spontaneous lung tumor incidence in this particular strain of mouse.

We went through chapter-by-chapter their detailed and scientifically well-prepared report, and we agreed that the difference between the control and test groups is not necessarily the result of oncogenic potency of Garlon.

The following details from the Company's report organized by Dr. R. J. Kociba are referenced:

1) The Use of Experimental Purpose of CDF/Cox Strain is Discontinued for the primary reason that it has quite frequently urinary system (obstruction) problem in old age.

2) The historical control group in various mouse strains are summarized in the following table:

TABLE 3
Historical Incidence Of
Spontaneous Lung Tumors for Various Mice Strains
(Excluding BALB/C, DBA/2+CDF_1/Cox Strains)

Mouse Strain	% of Incidence of Lung Tumors	
	MALE	FEMALE
Swiss CD-1	14	24
Swiss/ICR	4	6
ICR:Ha(ICR)	39	12
Charles River CD-1	21	5
Charles River CD-1	24	31
Tuck (TF_1)	12	15
TO	23	3
TO	16	20
Tuck	3	4
ASH/CSGI	27	7
ASH/CS_1	43	19
ASH/CS_1	43	29
ASH/CS_1	30	14
LP	5	4
129	0	1
CBA	0	0
$C_{57}BL/10$	0	3
C_3H	7	No Data
Swiss-Webster	10	14
$B_6C_3F_1$	18	4
$B_6C_3F_1$	13	2
$B_6C_3F_1$	18	4
$B_6C_3F_1$	4	8
$B_6C_3F_1$	10	12
$B_6C_3F_1$	14	4

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Table 3 (continued)

	% of Incidence of Lung Tumors	
	MALE	FEMALE
A		90
SWR		80

Both in male and female groups there is a greater variation in percent of incidence of lung tumors. Several of them are similar or higher, and some are lower, than the control group in this experiment.

3) Historical data in lung tumors in a related strain are illustrated in the following text:

TABLE 4

Control Group Data for Pulmonary Tumors in $B_6C_3F_1$ Mice (related to CDF/Cox)
From National Cancer Institute National Toxicity Program
Cancer Bioassay Draft Reports,
Cancer Bioassay Final Reports,
or Dow Chemical Toxicology Research Laboratory

% Incidence of Pulmonary Neoplasms

Study	MALE Adenoma/Carcinoma Total	FEMALE Adenoma/Carcinoma Total
2,4 Dimethoxyanaline Hydrochloride	20	5
Sodium Diethyldithiocarbamate	31	0
4-Nitro-o-Phenylene diamine	17	20
Calcium Cyanide	35	15
p-Cresidine	14	9
Nithiazide	15	19
Lead Dimethyl-dithiocarbamate	45	15
Sodium Alcohol	10	0
Sodium Docecyl Sulfate	4	2
HC Blue 1	12	8
HC Red 3	26	2
Ethyl Acrylate	16	4
Allyl Isovalerate	26	8
Geranyl Acetate	12	2
Benzyl Acetate	25	2
"A" Control 1	27	6
"A" Control 2	20	3
"B"	8	4
"C"	10	9

It can be concluded that there is great variation in incidence in spontaneous lung tumors when CDF/Cox-related strain of mice were used. In some experiments, the incidence is similar to this experiment; in others, it is higher or lower.

4) The "Concurrent Control Proportional Basis" total (adenoma and carcinoma) number of tumors in different test groups and concurrent control group does not show differences.

In the evaluation of this finding, I would like to make a quotation from the "Animal Data in Hazard Evaluation: Path and Pitfalls: Taskforce of Past President", Society of Toxicology F.A.A.T., 2:101, 1982:

> "If the incdence rate in the concurrent control group is lower than in the historical control groups, but the incidence rates in the treated groups are within the historical control range, the differences between treated and control groups are not biologically significant." But in this experiment the concurrent controls were not lower, only the study controls were lower than in the test groups.

5) There is no decrease in the latency of tumor appearance in the test groups compared to controls.

6) There is no increase in malignancy in tumor incidence in test groups compared to controls.

7) There is no difference in hyperplastic changes in the test groups and in controls.

8) There is no significant difference in survival rate among the groups.

9) It is generally expected that whenever an oncogenic agent affects test animals, a dose-related increase of incidence is present. This phenomenon is considered one of the strongest proofs of oncogenicity. In this mouse experiment, no significant difference can be observed among the test groups, both in males and females.

Giving careful evaluation of the results of different data and measuring the weight of differences between control and test groups and considering the results of historical and concurrent control data, it can be concluded that the oncogenic potential of "Garlon" can not be substantiated.

As a note, I also make reference to our report of May 6, 1982. This is important in consideration for the final conclusion.

a) For teratology study in a second species will be required.

b) Teratogenic Evaluation of Dowco 233 in the Rat.
NOEL= 200 mg/kg/day (HDT) (Fetotoxic effects at 200 mg/kg/day; regarded ossification of fetal skull bones at 200 mg/kg/day, and elevated incidence of sternbrae variations at 100 mg/kg/day dam treatment) Core-Minimum Data.

c) Mutagenicity Test on Triclopyr (Dowco 233) in Bacterial Systems. Rec-assay and Reversion Mutagenicity Tests - Negative. Acceptable Study.

d) Ames Metabolic Activation Test to Assess the Potential Mutagenic Effect of Dowco 233. No mutagenic potential. Core-Minimum Data.

e) No valid rat experiment is available for evaluation of Garlon at this time.